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Title: Nitroaromatic and Nitroamine Explosive Compounds by High Performance Liquid Chromatography (HPLC) [SW-846 8330A & 8330B]

Facility Distribution No. _____ **Distributed To:_____**

1.0 Scope and Application

1.1 This standard operating procedure (SOP) describes the determination of nitroaromatic and nitroamine explosive residues by high performance liquid chromatography (HPLC) using dual columns and dual UV wavelengths. This includes analysis in water, soil, and sediment matrices. The instrumental analysis is based on EPA Methods 8330A and 8330B.

1.2 This SOP does not include the extraction procedures. For those details, please refer to DV-OP-0017 *Solid Phase Extraction of Nitroaromatic and Nitroamine Explosive Compounds and Picric Acid from water samples by SW-846 3535A* and DV-OP-0018 *The Extraction of Nitroaromatic and Nitroamine Explosive Compounds and Picric Acid from Soil Samples by SW-846 8330A and 8330B.*

1.3 On occasion, clients may request modifications to this SOP. Requests for modifications must be received in writing and will be communicated to the laboratory through method comments in the LIMS. Significant method changes require a work instruction signed by both the client and TestAmerica Denver management and the Quality Assurance Manager. (See SOPs DV-QA-001P and DV-QA-0010.)

1.4 Application of 8330A versus 8330B

1.4.1 This procedure is for analysis by either Method 8330A or 8330B. The most important differences in the two source methods are the more rigorous sample collection and preparation measures in 8330B, which are designed to produce more representative results. The more rigorous 8330B process is specifically intended to complement the multi-increment field sampling process described in Appendix A of 8330B. If multi-increment or equivalent systematic sampling processes are not employed in the field, then the extra laboratory homogenization and subsampling effort 8330B requires (see details in DV-OP-0018) may add little or no improvement in the overall precision of results.

1.4.2 For soil analysis a sample size of 2 g is used for 8330A and a sample size of 10 grams is used for 8330B.

1.4.3 8330A only describes the cyano (CN) column for confirmation. 8330B gives the option of either cyano (CN) or pheny-hexyl columns for confirmation. Because it provides better sensitivity and resolution, TestAmerica Denver routinely confirms using the Phenomenex Luna Phenyl-Hexyl column for both methods.

1.4.4 8330B also added compounds to the potential analyte list. TestAmerica Denver offers all of the compounds shown in Appendix 1 of this SOP by both methods, except 3,5-dinitroaniline, which is only analyzed by 8330B.

1.5 Analytes, Matrix(s), and Reporting Limits

1.5.1 The list of analytes, CAS numbers, abbreviations and TA-Denver's standard reporting limits can be found in Appendix 1.

1.5.2 The working ranges of this method are as follows:

Analytes	8330A Soil (2g prep)	8330B Soil (10g prep)	Water
All Analytes except nitroglycerin and PETN	0.2 µg/g – 25 µg/g	0.08 µg/g – 10 µg/g	0.2 µg/L - 25 µg/L
Nitroglycerin and PETN	2.0 µg/g – 250 µg/g	0.80 µg/g – 100 µg/g	2.0 µg/L - 250 µg/L

2.0 Summary of Method

2.1 Instrument calibration is performed by external standardization using a minimum of five concentration levels.

2.2 An acetic acid and phosphate buffer in water / methanol gradient program is used for HPLC separation (see details in Section 7.8.10 and Appendix 4). Compounds are tentatively identified based on retention time and detection by the UV detector using the primary Agilent Poroshell 120 EC-C18 column. Confirmation is performed by the UV detector using a Phenomenex Luna Phenyl-Hexyl column (see Appendix 4 for instrument conditions).

3.0 Definitions

3.1 Explosives: As used in this SOP, the term "explosives" refers specifically to the analytes listed in Appendix 1. These include compounds that can be readily detonated with heat, shock, or ignition, such as nitroglycerin, RDX, and TNT. It also includes production by-products and degradation products of true explosives.

3.2 Definition of terms used in this SOP may be found in the Glossary section of the TestAmerica Denver Quality Assurance Manual (QAM) and in SOP QA-DV-003P.

4.0 Interferences

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines, causing misinterpretation of the chromatograms. All of these materials must be demonstrated to be free from interferences, under the conditions of the analysis, by running method blanks.

4.2 Contamination by carryover can occur when a low-concentration sample is analyzed immediately following a high-concentration sample. This potential is minimized by continuously flushing the needle with solvent. If contamination is suspected, the sample should be re-aliquoted and re-analyzed.

4.3 Co-elution of target analytes with non-target analytes can occur, resulting in false positives or biased high results.

4.4 Co-elution between target analytes can occur when high concentrations of individual compounds are present in samples, see Section 12.2.3.5 for details.

4.5 The inclusion of vegetation is not recommended given the nature of the detector and different uses the data will potentially support (USACE comment – Issue #306 TA Denver Audit Database; DOD/DOE QSM 5.0 and 5.1 both state to exclude vegetation).

4.6 Tetryl decomposes rapidly in methanol/water solutions, as well as with heat. All samples expected to contain tetryl should not be exposed to temperatures above room temperature. (Reference: EPA Method 8330A & 8330B, Section 4.3) Elution solvent for the SPE cartridges is also acidified to help preserve tetryl in sample extracts.

5.0 Safety

Employees must abide by the policies and procedures in the Environmental Health and Safety Manual (CW-E-M-001), Radiation Safety Manual (RSM) and this document. This procedure may involve hazardous material, operations and equipment. This SOP does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of the method to follow appropriate safety, waste disposal and health practices under the assumption that all samples and reagents are potentially hazardous. Safety glasses, gloves, lab coats and closed-toe, nonabsorbent shoes are a minimum.

5.1 Specific Safety Concerns or Requirements

5.1.1 Eye protection that satisfies ANSI Z87.1, laboratory coat, and nitrile or latex gloves must be worn while handling samples, standards, solvents, and reagents. Disposable gloves that have been contaminated will be removed and discarded; non-disposable gloves must be cleaned immediately.

5.1.2 If a sample is expected to have an explosive concentration ≥2% (but less than 10%), the EH&S Coordinator and Group Leader shall be notified before any work is performed. Additional safety precautions may be implemented as required due to high concentrations of explosives.

WARNING: Soil samples with explosive concentrations greater than 2% cannot be accepted by the laboratory unless they have a moisture content of 25% or greater. Under no circumstances shall a soil sample with an explosive concentration greater than 10% be accepted by the laboratory.

5.1.3 Soil samples with high concentrations (between 2 and 10%) of explosives should not be ground using a mortar and pestle. Visual observation of a

soil samples is important prior to grinding samples. Any samples containing metal fragments, powders, waxy appearing pieces, or other suspicious material should be brought to the attention of the Group Leader and the EH&S Coordinator before proceeding with the procedure. Bypassing the grinding step and proceeding to solvent dilution is an alternative for samples that are determined to be unsafe to grind.

5.2 Primary Materials Used

The following is a list of materials used in this method, which have a serious or significant hazard rating.

NOTE: This list does not contain all materials used in the method. The table contains a summary of the primary hazards listed in the SDS for each of the materials listed in the table.

A complete list of materials used in the method can be found in the reagent and materials section. Employees must review the information in the SDS for each material before using it for the first time or when there are major changes to the SDS.

MATERIAL [1]	HAZARDS	EXPOSURE LIMIT [2]	SIGNS AND SYMPTOMS OF EXPOSURE
Acetonitrile	Flammable Poison	40 ppm – TWA	Early symptoms may include nose and throat irritation, flushing of the face, and chest tightness. Prolonged exposure to high levels of vapors may cause formation of cyanide anions in the body.
Glacial Acetic Acid	Corrosive Poison Flammable Liquid and Vapor	10 ppm - TWA	Inhalation of concentrated vapors may cause serious damage to the lining of the nose, throat, and lungs. Breathing difficulties may occur. Can cause serious damage to skin, including redness, pain, and burns. Contact with eyes may cause severe damage followed by loss of sight.
Methanol	Flammable Poison Irritant	200 ppm - TWA	A slight irritant to the mucous membranes. Toxic effects are exerted upon the nervous system, particularly the optic nerve. Symptoms of overexposure may include headache, drowsiness, and dizziness. Methyl alcohol is a defatting agent and may cause the skin to be become dry and cracked. Skin absorption can occur, symptoms may parallel inhalation exposure. Irritant to the eyes.
Phosphoric Acid	Corrosive	1 ppm - TWA	Ingestion can cause severe burns to the throat, mouth, and stomach, abdominal pain and nausea. Severe exposures by ingestion can lead to shock, circulatory collapse, and death. Inhalation is not an expected hazard unless misted. Corrosive, contact with skin or eyes can cause redness, pain, severe burns, blurred vision, and permanent eye damage.
Sodium hydroxide	Corrosive Poison	2 mg/m^3	Effects from inhalation of dust or mist vary from mild irritation to serious damage of the upper respiratory tract, depending on severity of exposure. Symptoms may include sneezing, sore throat, runny nose. Contact with

MATERIAL [1]	HAZARDS	EXPOSURE LIMIT [2]	SIGNS AND SYMPTOMS OF EXPOSURE
			skin can cause irritation or severe burns and scarring with greater exposures. Causes irritation of eyes and can cause burns that may result in permanent impairment of vision, even blindness with greater exposures.
(1) Always add acid to water to prevent violent reactions. (2) Exposure limit refers to the OSHA regulatory exposure limit.			

6.0 Equipment and Supplies

6.1 Instrumentation

6.1.1 HPLC System

HPLC, equipped with a pump capable of achieving 6000 psi, a 100 µL loop injector, and a Diode Array Detector (DAD) or Multi-Wavelength Detector (MWD), Hewlett Packard Model 1100, or equivalent.

6.1.2 Primary Column: Reverse phase HPLC column, Agilent Poroshell 120, EC-C-18, 4.6mm x 150mm (2.7 µm) or equivalent.

6.1.3 Confirmation Column: Phenomenex Luna Phenyl-Hexyl reverse phase HPLC column, 15 cm x 4.6 mm (3 µm) or equivalent.

6.1.4 Hewlett Packard HPLC Chem Station for instrument control.

6.2 Supplies

6.2.1 Glass vials, various sizes.

6.2.1.1 Amber glass, 8.0 mL and 12.0 mL, with Teflon-lined screw caps, for the storage of standards.

6.2.1.2 Crimp-top vial with caps for analysis, 1.8 mL.

6.2.2 Disposable pipettes, used for non-quantitative transfers only.

6.2.3 Volumetric flasks, various sizes.

6.2.4 Hamilton syringes, various sizes.

6.3 Computer Software and Hardware

Please refer to the master list of documents and software located on R:\QA\Read\Master List of Documents\Master List of Documents, Software and

Hardware.xls or current revision for the current software and hardware to be used for data processing.

7.0 Reagents and Standards

7.1 Stock Standards

7.1.1 Stock standards are purchased as certified solutions or prepared from 100%, neat materials. Stock standard solutions are stored at -10 ℃ to -20 ℃, or per vendor instructions. All stock standard s must be protected from light and should be brought to room temperature before using.

7.1.2 Stock standard solutions must be replaced after 1 year or sooner if comparison with check standards prepared from an independent source indicates a problem. Expiration times for all standards are measured from the time the standard is prepared or from the time that the standard ampoule is opened, if the standard is supplied in a sealed ampoule.

7.1.3 3,5-Dinitroaniline is purchased at a concentration of 100 µg/mL in acetonitrile, equivalent to the High-Level Calibration Mix. This standard is added directly to the Intermediate Level Calibration Standard. (Section 7.4).

7.1.4 PETN and Nitroglycerin are purchased as separate individual standards at a concentration of 1000 µg/mL in acetonitrile. These standards are added directly to the Intermediate Level Calibration Standard (Section 7.4).

7.1.5 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene are purchased as separate individual standards at a concentration of 100 µg/mL in acetonitrile, equivalent to the High-Level Calibration Mix. These standards are added directly to the Intermediate Level Calibration Standard (Section 7.4).

7.1.6 MNX is purchased as a pre-weighed neat standard and comes in powder form. A quantitative transfer is performed and the final concentration calculated for the high level MNX standard. The volume of this standard used for intermediate stock preparation will vary depending on the initial weight of MNX sent. This is the only known source of MNX and we do not currently have a second source for this compound.

7.2 Volume Measurements for all Standards Preparation

The volume of stock and intermediate standard solutions used in subsequent dilutions is measured using Hamilton syringes appropriate for the volume being measured and accurate to 2%. Standards are prepared either by (1) using a syringe to measure the standard solution and bringing to volume with the appropriate solvent in a Class A volumetric flask, or by (2) measuring the volumes of both the standard solution and the solvent using a calibrated syringe or Class A pipette and combining them in a vial.

7.3 High-Level Calibration Mix, Prepared from Stock Standards

This high level standard must be replaced every 1 year or sooner if comparison with check standards prepared from independent sources indicates a problem.

A solution is prepared to contain most standard analytes at a concentration of 100 µg/mL each in acetonitrile (see details in standards database instructions). Nitroglycerin, PETN, 3,5-Dinitroaniline, 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene are purchased as separate stock standards, and are not included in this mixture. They are added to the intermediate level standards in section 7.4.

7.4 Intermediate Level Calibration Standards

8330IntermStk: Prepare a 20 µg/mL solution (nitroglycerin and PETN at 200 µg/mL) from the high-level calibration mix (see Section 7.3) by diluting 1.0 ± 0.02 mL of the High-Level Calibration mix along with 1.0 ± 0.02 mL each of stock standards of nitroglycerin and PETN to a final volume of 5.0 mL in acetonitrile. The shelf life of this material is 6 months.

8330_ADDs: Prepare a 20 µg/mL solution from stock standards by diluting 1.0 ± 0.02 mL each of stock standards of 3,5 -dinitroaniline, 2,4-diamino-6-nitrotoluene, and 2,6-diamino-4-nitrotoluene to a final volume of 5.0 mL in acetonitrile. The shelf life of this material is 6 months.

7.5 Working Level Standards for Calibration Curve

Prepare calibration standards by diluting the intermediate standard solutions as shown in the table below using the 75%:25% (v/v) acidic water:ACN solution (described in Section 7.8.7). These standards must be prepared fresh on the day of calibration and refrigerated if not used immediately. All volumes are measured using the appropriately sized Hamilton syringe.

7.5.1 On the primary (C18) column, the 8330IntermStk and 8330_ADDs standards must be calibrated using separate sets of calibration standards due to co-elutions with other target compounds. The intermediate standards are prepared at the same concentration; therefore, the calibrations are made with the same volumes of intermediate stock and solvent in the following table.

7.5.2 On the confirmation (phenyl-hexyl) column, the intermediate full-list and intermediate 3,5-dinitroaniline stocks can be combined into the same calibration standards and follow the “Confirmation (Phenyl-Hexyl) Column Calibration” recipes in the following table.

Recommended Calibration Levels

Calibration Level	Final Concentration (µg/mL)		Primary (C18) Column Calibration		Confirmation (Phenyl-Hexyl) Column Calibration	
	Standard Analytes	***NG & PETN***	***Vol. Intermediate (µL)***	***Vol. Solvent (µL)***	***Vol. EACH Intermediate (µL)***	***Vol. Solvent (µL)***
8	2.5	25.0	125 ± 1	875 ± 9	125 ± 1	750 ± 9
7	1.0	10.0	50 ± 0.5	950 ± 10	50 ± 0.5	900 ± 10
6	0.7	7.0	35 ± 0.4	965 ± 10	35 ± 0.4	930 ± 10
5	0.4	4.0	20 ± 0.2	980 ± 10	20 ± 0.2	960 ± 10
4*	0.25	2.5	12.5 ± 0.1	988 ± 10	12.5 ± 0.1	975 ± 10
3	0.1	1.0	5 ± 0.05	995 ± 10	5 ± 0.05	990 ± 10
2	0.05	0.5	2.5 ± 0.02	998 ± 10	2.5 ± 0.02	995 ± 10
1	0.02	0.2	20 ± 0.1 µL of Level 7	980 ± 10	20 ± 0.1 µL of Level 7	980 ± 10
*** Level 4 concentration is used for the daily and continuing calibrations.**						

7.6 Extractions Standards

7.6.1 LCS Spike Solution

The LCS spike solution is prepared at a working level concentration of 10 µg/mL (nitroglycerin and PETN at 100 µg/mL) in acetonitrile. This standard is stored in a freezer at –20℃ to –10℃ and given a six-month expiration date. The standard is allowed to come to room temperature before use and returned to the freezer as soon as possible.

This standard contains all explosives target compounds except 3,5-dinitroaniline, 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene.

7.6.2 3,5-Dinitroaniline LCS Solution

The 3,5-DNA LCS solution is prepared at a working level concentration of 10 µg/mL in acetonitrile. This standard is stored in a freezer at –20℃ to –10℃ and given a six-month expiration date. The standard is allowed to come to room temperature before use and returned to the freezer as soon as possible. This standard is used only for method 8330B and is spiked into separate LCS and MS/MSD samples. This compound cannot be completely resolved from tetryl and nitrobenzene on the primary column.

7.6.3 Diamino LCS Solution

The Diamino LCS solution is prepared at a working level concentration of 10 µg/mL in acetonitrile of 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene. This standard is stored in a freezer at –20°C to –10°C and given a six-month expiration date. The standard is allowed to come to room temperature before use and returned to the freezer as soon as possible. This standard is only added when these compounds are specifically requested by the client. They are added to a separate LCS/MS/MSD. They are spiked into the same LCS/MS/MSD as 3,5 DNA if the client requests all three compounds.

7.6.4 Working Level Surrogate (1,2-Dinitrobenzene) Solution

The 8330 surrogate solution is prepared at a working level concentration of 10 µg/mL in acetonitrile. This standard is stored in a freezer at –20°C to –10°C and given a six-month expiration date. The standard is allowed to come to room temperature before use and returned to the freezer as soon as possible.

7.7 Second Source Initial Calibration Verification Solution

The second source standard must be obtained from a different source than the standards used for initial calibration. This standard is used to verify the accuracy of the calibration standards.

NOTE: There is currently no second source available for MNX.

7.7.1 Working-Level Second Source Mix

Prepare a solution containing all compounds except 3,5-dinitroaniline, 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene at a concentration of 0.40 µg/mL (nitroglycerin and PETN at 4.0 µg/mL). A separate solution containing 3,5-dinitroaniline, 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene is prepared at a concentration of 0.40 µg/mL when analyzing on the primary (C18) column; the standards can be combined for analysis on the confirmation (phenyl-hexyl) column. These solutions are prepared using the 75%:25% (v/v) acidic water:ACN solution (described in Section 7.8.7). These standards must be prepared fresh on the day of calibration. All volumes are measured using the appropriately sized Hamilton syringe.

7.8 Reagents

7.8.1 Reagent Water

For method blanks and laboratory control samples reagent water is generated by an ELGA water purification system. The performance of the

water polishing system is checked daily and recorded per SOP DV-QA-0026.

7.8.2 HPLC Grade Water

7.8.3 Acetonitrile, CH_3CN (ACN) - HPLC grade

7.8.4 Methanol - HPLC grade

7.8.5 Glacial Acetic Acid – Reagent Grade

7.8.6 85% Phosphoric acid, H_3PO_4 – Reagent Grade.

7.8.7 Acidified Water (75%) : Acetonitrile Solution (25%)

TALS Reagent: 8330AcidH2O

Take 250 mL of acetonitrile (ACN), and bring to 1.0 L with Elga or HPLC-grade water. Acidify the solution to a pH of approximately 3 by adding 20 drops (1 mL) of 85% phosphoric acid (H_3PO_4).

7.8.8 Acidified Calcium Chloride, $CaCl_2$ Solution, 5 g/L

TALS Reagent: CaCL2 Sol

Place 5 ± 0.05 g of reagent grade $CaCl_2$ into a one-liter volumetric flask containing approximately 500 mL of deionized water. Swirl the solution until the $CaCl_2$ is dissolved. Add approximately 1 mL of 85% H_3PO_4 to acidify the solution and bring to volume with deionized water.

7.8.9 Sodium Phosphate Buffer Stock

TALS Reagent: 8330bufferstk

Slowly add 115.29 grams of 85% phosphoric acid (molecular weight = 97.9924 g/mol) to approximately 500 mL of water in a 1 L beaker. Place a stir bar in the beaker and the beaker in an ice bath on top of a magnetic stirrer. Slowly add 150 mL of 10 M (10 M = 10 N) sodium hydroxide, allowing time for the mixture to cool down between additions. Transfer to a 1 L volumetric flask and bring to volume with Elga or HPLC-grade water. The final pH of this solution should be 7.2.

7.8.10 Buffer Eluents for Analysis:

Make up the HPLC eluents for each column as described in Sections 7.8.10.1 and 7.8.10.2. The pH of the working eluents *must* be modified by the analyst by changing the volume of glacial acetic acid added to ensure compound resolution. This is particularly necessary when the column is

replaced, to ensure that picric acid does not co-elute with any other target compound. Increasing the concentration (or volume added) of glacial acetic acid will result in greater retention of picric acid. The eluent can only be adjusted at the start of an initial calibration, not with the CCVs. The calculated retention time windows should account for the drift of any specific analyte.

7.8.10.1 Working Eluent for Primary (C18) Column:

Combine 1 L of water, 1 mL of Sodium Phosphate Buffer Stock solution (Section 7.8.9), and 50 µL of Glacial Acetic acid to adjust the pH of the buffer to approximately 6.5. Make fresh at least weekly or more often as needed.

7.8.10.2 Working Eluent for Confirmation (Phenyl-Hexyl) Column:

Combine 1 L of water, 2 mL of Sodium Phosphate Buffer Stock solution (Section 7.8.9), and enough glacial acetic acid (approximately 65-95 µL) so that picric acid does not co-elute with MNX or RDX. The pH of the buffer should be adjusted to approximately 6.5. Make fresh before each run.

8.0 Sample Collection, Preservation, Shipment and Storage

8.1 Aqueous Samples

Water samples should be collected in duplicate 500 mL amber glass bottles with Teflon-lined caps.

8.2 Soil and Sediment Samples

8.2.1 For method 8330A, soil samples should be collected in eight-ounce wide mouth jars with Teflon-lined caps.

8.2.2 For method 8330B, it is not uncommon to receive samples of 1 kg or more. Samples may be shipped in wide mouth jars or clean plastic bags.

8.3 Samples and sample extracts must be stored in amber glass containers at ≤ 6℃ from the time of collection through analysis, except during drying.

8.4 Soil and sediment samples should be air dried at ambient temperature until dry enough to sieve. See DV-OP-0018 for details. Once the sample is air dried, the sample can be stored at room temperature.

Matrix	Sample Container	Min. Sample Size	Preservation	Holding Time	Reference
Water	Amber glass	1 Liter (2 x 500 mL)	Cool; ≤ 6°C	7 Days to Extraction 40 Days to Analysis	SW846 8330A
Soil	Glass / plastic	4 grams (8330A) / up to 1 kg (8330B)	Cool; ≤ 6°C	14 Days to Extraction 40 Days to Analysis	SW846 8330A/B

9.0 Quality Control

9.1 The minimum quality controls (QC), acceptance criteria, and corrective actions are described in this section. When processing samples in the laboratory, use the LIMS Method Comments to determine specific QC requirements that apply.

9.1.1 The laboratory's standard QC requirements, the process of establishing control limits, and the use of control charts are described more completely in TestAmerica Denver policy DV-QA-003P, Quality Assurance Program.

9.1.2 Specific QC requirements for Federal programs, e.g., Department of Defense (DoD), Department of Energy (DOE), etc., are described in TestAmerica Denver policy DV-QA-024P, Requirements for Federal Programs. This procedure meets all criteria for DoD/DOE QSM 5.0 and 5.1 unless otherwise stated.

9.1.3 Project-specific requirements can override the requirements presented in this section when there is a written agreement between the laboratory and the client, and the source of those requirements should be described in the project documents. Project-specific requirements are communicated to the analyst via Method Comments in the LIMS and the Quality Assurance Summaries (QAS) in the public folders.

9.1.4 Any QC result that fails to meet control criteria must be documented in a Nonconformance Memo (NCM). The NCM is automatically sent to the laboratory Project Manager by e-mail so that the client can be notified as appropriate. The QA group periodically reviews NCMs for potential trends. The NCM process is described in more detail in SOP DV-QA-0031. This is in addition to the corrective actions described in the following sections.

9.2 Batch Definition

Batches are defined at the sample preparation stage. The batch is a set of up to 20 samples of the same matrix, plus required QC samples, processed using the

same procedures and reagents within the same time period. Batches should be kept together through the whole analytical process as far as possible, but it is not mandatory to analyze prepared extracts on the same instrument or in the same sequence. The method blank must be run on each column on which the associated samples are analyzed. See QC Policy DV-QA-003P for further details.

9.3 Method Blank (MB)

A method blank (MB) must be prepared and analyzed with each batch of samples. The MB consists of reagent water for aqueous samples, and Ottawa sand for soil samples, with surrogates added. The MB is created at the time of extraction after the samples have been dried, sieved, and ground and is then carried through all extraction and analysis steps. The method blank is used to identify any system and process interferences or contamination of the analytical system that may lead to the reporting of elevated analyte concentrations or false-positive data. See section 9.9 for the use of the grinding blank for Method 8330B solids samples.

Acceptance Criteria: The MB must not contain any analyte of interest at or above one-half the RL or at or above 10% of the measured concentration of that analyte in the associated samples, whichever is higher.

MBs are evaluated on each column on which associated samples are analyzed, when confirmation data are required. If there is a detection on either column in the MB, then detections for that target compound are suspect in associated samples. See Appendix 4 for guidance on interpretation of confirmation data to assess acceptance of the MB.

Corrective Action: If the method blank does not meet the acceptance criteria, the source of contamination must be investigated and measures taken to correct, minimize, or eliminate the problem. Reanalyze and/or reprepare all samples associated with a failed method blank.

If the MB acceptance criteria are not met and re-preparation and reanalysis are not possible, then the sample data associated with the unacceptable MB must be qualified. This nonconformance must be addressed in the project or case narrative and the client must be notified.

9.4 Laboratory Control Sample (LCS)

One LCS must be analyzed with each batch of samples (up to 20 samples). The LCS must contain specified analytes of interest and must be carried through the entire analytical procedure. For water samples, the LCS is prepared by spiking the analytes of interest into reagent water. For soil samples, the LCS is prepared by

spiking the analytes of interest into Ottawa sand. The LCS is created at the time of sample extraction after the samples have been dried, sieved and ground. The LCS is used to monitor the accuracy of the analytical process. On-going monitoring of the LCS results provides evidence that the laboratory is performing the method within acceptable accuracy and precision guidelines. The LCS must be analyzed on the confirmation column. If there is insufficient volume to prepare an MS/MSD, and LCSD must also be prepared and analyzed.

Acceptance Criteria: The LCS recovery for each spiked analyte must be within established control limits. Laboratory default control limits are calculated as ± 3 standard deviations around the mean of historical data, as described in SOP DV-QA-003P. For DOD/DOE work, QSM limits are applied unless project specific limits are requested by the client. When no QSM limits are available, laboratory historical limits are applied. Control limits are maintained in the LIMS.

In accordance with the TNI 2009 Standard a marginal exceedance within ± 4 standard deviations is allowed for one of the analytes. This is based on the number of analytes typically spiked for this method, which is between 11 and 30. These acceptance criteria may be superseded by project-specific limits, as applicable.

Corrective Action: If recoveries for all spiked analytes are not within the acceptance limits, including the one allowed marginal exceedance, the analytical system is out of control and corrective action must occur. Generally this requires re-extraction and reanalysis of all associated samples. If the LCS is biased high and all associated samples are ND, not detected, it may be possible to report results with an NCM (see requirements for individual programs and clients).

9.5 Matrix Spike Sample (MS) and Matrix Spike Duplicate (MSD)

A matrix spike (MS) is a field sample to which known concentrations of target analytes have been added. A matrix spike duplicate (MSD) is a second aliquot of the same sample (spiked identically as the MS) prepared and analyzed along with the sample and matrix spike. The soil matrix spikes are created at the time of extraction. Spikes and surrogate compounds are added after the sample has been dried, sieved, and ground. One MS/MSD pair must be processed for each preparation batch (up to 20 samples). The MS/MSD results are used to determine the effect of a matrix on the precision and accuracy of the analytical process. Samples identified as field blanks, equipment blanks, or rinse blanks cannot be used for MS/MSD analysis.

Acceptance Criteria: The spike recoveries must fall within established control limits. The relative percent difference (RPD) between the MS and MSD must be less than or equal to the established RPD limit. LCS limits are used for MS/MSD evaluation. Control limits are maintained in the LIMS. For DoD/DOE work, QSM limits are applied if available unless project specific limits are requested by the client. The RPD limit for DoD/DOE QSM 5.0 and 5.1 work is 30% for Method 8330A and 20% for Method 8330B.

Corrective Action: The information obtained from MS data are sample/matrix specific and are not normally used to determine the validity of the entire batch. If the MS and/or MSD recovery falls outside of the established control limits, the bracketing CCV and batch LCS recoveries must be within control limits in order to accept results for the associated samples. The following corrective actions are required for MS/MSD recovery failures to rule out lab error:

- Check calculation and instrument performance;
- Verify, if possible, that the MS and MSD were spiked correctly (e.g., very low or very high recoveries);
- Consider objective evidence of matrix interference (e.g., heterogeneous sample, interfering peaks seen on chromatograms, or interference demonstrated by prior analyses);
- Flag the data for any results outside of acceptance limits.
- For any single RPD failure, check calculations; verify, if possible, that the MS and MSD were spiked correctly; check instrument performance; consider objective evidence of matrix interference or sample inhomogeneity; and flag the data.
- If both the parent sample and associated matrix spike results are over range the parent and the spikes shall be diluted by the same amount and the results from the reanalysis reported for both. If the analyte concentration in the parent sample is greater than four times the concentration of spike added, then spike recovery results are not compared to control limits, and the recovery is either reported as “NC” (not calculated) or with a qualifier flag to indicate that the spike was less than four times the analyte concentration in the sample. If the dilution will cause the spike to be less than two times the reporting limit, the MS/MSD do not need to be

diluted and the recovery reported as "NC" (not calculated).

- For MS/MSD that serve as batch QC, if the parent sample result is within the calibration range and the MS/MSD results are above the calibration range, the results are reported with the MS/MSD result being flagged as an over-range measurement (e.g., the E-flag qualifier).
- If the MS/MSD are client requested, the parent sample result is within calibration range and the MS/MSD results are above the calibration range, the sample and spike should be diluted, keeping in mind that we need to assess whether or not the dilution will best serve the client's needs. Consult with the PM as needed. Both the parent sample and MS/MSD samples must have the same dilution factor. Some EDDs do not accept data that are at different dilution factors.
- If the native analyte concentration in the MS/MSD sample exceeds 4 times the spike level for that analyte, the recovery data are reported as NC (i.e., not calculated) and the appropriate qualifier flags are added.

NOTE: See Denver Policy Memorandum P16-001 and Corporate Policy Memorandum CA-Q-QM-013 for more detail.

NOTE: Some client programs require reanalysis to confirm matrix interferences. Check special project requirements for this corrective action.

9.6 Surrogates

Each calibration standard, field sample, and QC sample is spiked with the surrogate compound 1,2-dinitrobenzene. The surrogate is added to field samples and QC samples before the first extraction step for all matrices.

Acceptance Criteria: The recovery of the surrogate must fall within established statistical limits, which are based on historical data.

Corrective Action: If recoveries for surrogates in blanks or LCSs are outside of the control limits, check for calculation or instrument problems and reprepare and reanalyze the associated samples.

For samples with failing surrogate recoveries the decision to reanalyze or flag the data should be made as required by the project.

If matrix interference is obvious from observation of chromatograms or other objective evidence, reanalysis is unlikely to produce new or more useful information. If the matrix interference is not obvious from the initial analysis, it is only necessary to reprepare and reanalyze a sample once to demonstrate that poor surrogate recovery is due to matrix effect, so long as the extraction/instrument system is proven to be working properly.

9.7 Sample Duplicate

NOTE: Method 8330B requires the preparation of both a soil duplicate and a soil triplicate. See Section 9.8.

Although not typically required for organic analyses, a duplicate sample may be required for project-specific quality control. In this case, a sample duplicate is a second aliquot of one of the samples in the batch. Field blanks cannot be used for duplicate testing. The results for duplicates are reported separately, and cannot be averaged when reporting results. Sample duplicate results are used to evaluate the precision of the method. As such, results should be greater than or equal to the RL for a valid statistical comparison.

Acceptance Criteria: The RPD between the sample and the sample duplicate results must be less than the established limit.

Corrective Action: Results for samples that do not meet acceptance limits, particularly if due to difficulties in subsampling, shall be discussed in the final report case narrative, after client notification and agreement.

9.8 Sample Replicates

Replicate analyses are not part of the laboratories standard quality control samples. Method 8330B requires the preparation and analysis of sample duplicate and triplicate for soil samples ground by the ring and puck or ball mill. The lab will extract triplicate aliquots after grinding on the client designated sample. If a sample is not designated by the client, the lab will select the sample. The lab will determine the %RSD as defined below. Results for the %RSD as well as the individual replicate results will be reported to the client. The %RSD for results above the LOQ must be $\leq$ 20%, including DoD/DOE samples.

The percent relative standard deviation (%RSD) is calculated as follows:

$$\%RSD = \frac{s}{\bar{C}} \times 100\% \qquad \textit{Equation 1}$$

Where s is the standard deviation of the average concentration ($\bar{C}$) and is calculated as follows:

$$s = \sqrt{\frac{\sum_{i=1}^{n} (C_i - \bar{C})^2}{n-1}} \qquad \textit{Equation 2}$$

In the event that the laboratory is requested to perform the evaluation of field replicate precision, three field replicates designated by the client will be processed through the entire homogenization and extraction steps. The %RSD for these replicates will be calculated as indicated above and reported to the client.

9.9 Grinding Blank (GB)

Refer to SOP DV-OP-0018 for details on how the grinding blanks for soils by method 8330B are prepared. The laboratory composites the grinding blanks to prepare and analyze one grinding blank per batch, consistent with DOD/DOE QSM 5.0. DOD/DOE QSM 5.1 requires only one grinding blank per batch of samples, processed after the LCS (if ground) or after a client identified sample with known contamination, or at the end of the batch.

Acceptance Criteria: The grinding blank must not contain any analyte of interest at or above one-half of the RL or above 10% of the measured concentration of that analyte in the associated samples, whichever is higher.

Corrective Action: Per the method, the entire client sample is ground initially. Therefore, it is not possible to re-grind client samples if the grinding blank fails. Sample results will be reported with an NCM (see requirements for individual programs).

If the composite grinding blank results are greater than the acceptance limits, then the individual grinding blanks will be extracted and analyzed to determine when the contamination occurred and exactly which samples were affected. The potential carry-over between samples associated with a contaminated grinding blank producing positive results for the same contaminant must be described in a non-conformance memo and discussed in the final report case narrative.

9.10 Grinding LCS (LCSSRM)

Refer to SOP DV-OP-0018 for details on how the grinding LCS for soils by method 8330B is prepared. The grinding LCS is spiked by an outside vendor, and then ground with the associated samples. One grinding LCS per batch is required.

Acceptance Criteria: The grinding LCS recovery for each spiked analyte must be within established control limits. Control limits are maintained in the LIMS.

Corrective Action: Per the method, the entire client sample is ground initially. Therefore, it is not possible to re-grind client samples if the grinding LCS fails. Sample results will be reported with an NCM (see requirements for individual programs).

If the surrogate compound recovers below control limits, or every compound recovers outside of control limits, but at approximately the same percentage, this could be an indication of a bad extraction (post-grinding). Associated samples will be sent for re-extraction and re-analysis.

10.0 Procedure

10.1 One-time procedural variations are allowed only if deemed necessary in the professional judgment of supervision to accommodate variation in sample matrix, radioactivity, chemistry, sample size, or other parameters. Any variation in procedure shall be completely documented using an NCM. The NCM is automatically sent to the laboratory Project Manager by e-mail so that the client can be notified as appropriate. The QA group periodically reviews NCMs for potential trends. The NCM process is described in more detail in SOP DV-QA-0031. The NCM shall be filed in the project file and addressed in the case narrative.

10.2 Any deviations from this procedure identified after the work has been completed must be documented in an NCM, with a cause and corrective action described.

10.3 Extraction of Water Samples – *Please reference DV-OP-0017 for details*

Samples are extracted using a 500 mL initial volume and are concentrated to a 5 mL final volume.

10.4 Extraction of Soil Samples – *Please reference DV-OP-0018 for details*

10.4.1 Samples for method 8330A are extracted using a 2 g initial weight and delivered to the analytical group with a 10 mL final volume. Samples for method 8330B are extracted using a 10 g initial weight and delivered to the analytical group with a 20 mL final volume.

10.4.2 TALS prep batches for soils will show a final volume of 20 mL for 8330A extracts, and 40 mL for 8330B extracts. These final volumes include the 1:1 dilutions with the acidified calcium chloride done by the analyst prior to instrument analysis. (See Section 10.5.2.)

10.5 HPLC Analysis

10.5.1 HPLC Startup: All electronic equipment should be allowed to warm up for 30 minutes. During this period, at least 15 void volumes of mobile phase are passed through the column. Continue until the detector's baseline has stabilized.

10.5.2 Prior to analysis, soil extracts (or a portion of the extract) must be diluted exactly 1:1 with the acidified calcium chloride solution that is described in section 7.8.8.

10.5.3 Analyze the samples using the chromatographic conditions given in Appendix 5. All positive measurements above the method detection limit observed on the primary C18 column are confirmed by injection of the sample extract onto the confirmation Phenyl-Hexyl column. The MB and LCS must be analyzed on the confirmation column if samples are analyzed on that column. The MS and MSD must be analyzed on the confirmation column if the parent sample is analyzed on the confirmation column. Many EDDs require the parent and MS/MSD results be from the same analytical batch. For DoD/DOE work, calibration and QC criteria are the same as for primary column analysis. QC must pass on both columns when confirmation analysis is performed for DoD or DOE.

10.5.4 Analytes are introduced by direct injection of the extract. Samples, standards, and QC samples must be introduced using the same procedure.

10.5.5 It has been demonstrated that water samples with total concentrations of 16,000 µg/L can yield low recoveries due to saturation of the extraction cartridge. The client should be contacted to determine if re-extraction using a smaller sample aliquot size is required for samples with concentrations in this range. The low extraction recovery may meet the client's action limit, such that a re-extraction may not be necessary to prevent possible laboratory contamination.

10.5.6 Analytical Sequence

10.5.6.1 The analytical sequence starts with either an initial calibration or a Continuing Calibration Verification (CCV). If the sequence begins with a CCV, the center of the retention time window is set based on the initial CCV in the sequence. Do not reset the retention times with the bracketing CCV.

10.5.6.2 The CCV includes analyzing standards that contain all target analytes. If 3,5-dinitroaniline, 2,4-diamino-6-nitrotoluene and

2,6-diamino-4-nitrotoluene are not target compounds, it is not required to analyze a CCV for these analytes.

10.5.6.3 If there is a break in the analytical sequence greater than 12 hours since the analysis of a CCV standard, a new CCV standard must be analyzed before proceeding with the sequence.

10.5.7 Retention Time Windows

10.5.7.1 Retention time windows must be determined for all analytes. Make an injection of all analytes of interest each day for a three-day period. Calculate the standard deviation of the three retention times for each analyte (relative retention times may also be used). The width of the retention time window for each analyte is defined as ± three times the standard deviation.

NOTE: Determination of retention time windows using the 72-hour study is required for DoD/DOE work. A retention time window report can be generated in the Control Chart Module in TALS.

10.5.7.2 The chromatograms in Appendices 5 and 6 summarize the estimated retention times on both the C18 and Phenyl-Hexyl columns for many of the compounds analyzed using this method.

10.5.7.3 The center of the retention time window is the retention time from the last of the three standards. The centers of the windows are updated with the mid-point of the initial calibration, and each subsequent initial CCV (i.e., the CCV that begins the analytical sequence.) The widths of the windows will remain the same until new windows are generated following the installation of a new column.

10.5.7.4 If the retention time window, as calculated above, is less than ± 0.035 minutes for the C18 column or less than ± 0.07 minutes for the phenyl hexyl column, use ± 0.035 or ± 0.07 minutes as the retention time window. This allows for slight variations in retention times caused by sample matrix.

10.5.7.5 The laboratory must calculate new retention time windows each time a new column is installed or at least annually. Until these standards have been run on the new column, the retention time windows from the old column may be used, but updated with the retention times from the new initial calibration.

10.5.8 Daily Retention Time Windows

The center of the retention time window is adjusted to the retention time of each analyte, as determined in each initial calibration or each initial CCV.

Note: Chromatographic conditions, including the exact makeup of the eluent are determined at the time of the initial calibration and shall not be changed until the next initial calibration.

Corrective Action:

If there are shifts in retention times for target compounds between CCVs that are outside the established retention time window (see Section 10.5.7), all samples analyzed after the last compliant standard must be reanalyzed unless the following conditions are met for any compound that elutes outside the retention time window:

- The retention time of that compound in the standard must be within the retention time range equal to twice the original window, as determined by the opening CCV of a bracket, and
- The retention time of the compound must be shifted in the same direction as the surrogate and by approximately the same amount.

If these two conditions are met, reset the window and reprocess the data.

10.6 Calibration Range and Dilutions

10.6.1 If the concentration of any analyte exceeds the working range as defined by the calibration standards, then the sample must be diluted and reanalyzed. Dilutions should target the most concentrated analyte in the upper half (between the CCV and highest standard) of the calibration range.

10.6.2 Samples that are analyzed immediately following a sample with an unusually high concentration of explosives must be evaluated for carryover. The potential for carryover is minimized in the analytical system by continuously flushing the HPLC needle with solvent. If contamination is suspected, the sample should be re-aliquoted and re-analyzed.

10.6.3 Guidance for Dilutions Due to Matrix Interference:

It may also be necessary to dilute samples because of matrix interferences. If the sample is initially run at a dilution and only minor matrix peaks are present, then the sample should be reanalyzed at a more concentrated dilution. Analyst judgment is required to determine the most concentrated

dilution that will not result in instrument contamination. Ideally, the dilution chosen will make the response of the matrix interferences equal to approximately half the response of the mid-level calibration standard.

10.6.4 Reporting Dilutions

Some projects require reporting of multiple dilutions (check method comments in the LIMS). In other cases, the lowest dilution with no target compounds above the calibration range will be reported. In general, a maximum of two dilutions will be reported; one at the lowest dilution and one in which the most concentrated target analyte is in the upper half of the calibration range.

10.7 Instrument Maintenance and Troubleshooting

10.7.1 Minor instrument maintenance may include back flushing the column, changing the guard cartridge, and changing the frit on the front end of the column.

10.7.2 The solvent channel on which the buffer is run should be rinsed weekly with pure water followed by pure methanol and finally pure water again before reloading the buffer in order to prevent the buildup of salts and prevent bacterial growth in the system.

10.7.3 A cleanup method is provided in the instrument software to remove the buffer from the column. It should be run as the last injection of any run sequence. The buffer solution should not be left on the column for extended periods when the instrument is not in use or decreased column lifetime will be observed.

10.7.4 Noisy baseline, particularly noticeable in RL level standards and MDLV samples, is normally due to a noisy UV Lamp. If the noise is sufficient to interfere with the quantitation of these samples, the lamp should be replaced and the instrument recalibrated. Less commonly, noisy baselines are the result of dirty flow cell windows, which should be cleaned or replaced according the manufacturer's instructions.

10.7.5 Unstable retention times are normally due to a malfunction somewhere in the flow path of the instrument. Likely sources are the active inlet valve, outlet ball valve, multichannel gradient valve or purge valve. Dirty solvent inlet filters can starve the pump and may also result in unstable retention times.

11.0 Instrument Calibration

11.1 Detailed calibration equations can be found in the corporate SOP CA-Q-P-003, *Calibration Curves and the Selection of Calibration Points* and under the public folder, Arizona Calibration Training.

11.2 Instrument QC

11.2.1 External calibration is used for this analysis. Prepare standards containing each analyte of interest at a minimum of five concentration levels. The low level standard should be at or below the reporting limit. The other standards define the working range of the detector. Recommended calibration levels are given in Appendix 2.

11.2.2 A new calibration curve must be generated after major changes to the system or when the continuing calibration criteria cannot be met. Major changes include a new column and any changes in instrument operating parameters (including solvent flows, replacement of a detector lamp, replacement of the flow cell windows, etc.).

11.2.3 With the exception noted in Section 11.2.4 below, it is NOT acceptable to remove points from the calibration curve for the purpose of meeting criteria, unless the points are the highest or lowest on the curve AND the reporting limit and/or linear range is adjusted accordingly. In any event, at least 5 points must be included in a linear calibration curve and at least 6 points for a second order calibration curve.

11.2.4 A level may be removed from the calibration if the reason can be clearly documented (i.e., a broken vial or an injection error). A minimum of five levels must remain in the calibration for a linear model and six for a second order model. The documentation must be retained with the initial calibration.

11.3 Initial Calibration

Calibrations are modeled either as average response factors or as calibration curves, using a systematic approach to selecting the optimum calibration function in order as follows. When calibration acceptance criteria cannot be met for a model, appropriate corrective action must be taken. This may include processing the data using another model, instrument maintenance and or re-preparation of standards followed by recalibration.

11.3.1 The following requirements must be met for any calibration to be used:

11.3.1.1 Response must increase with increasing concentration.

11.3.1.2 Calibration curves will not be forced through the origin.

11.3.1.3 The absolute value of the intercept of the curve at zero response should ideally be less than the MDL for the analyte. At a minimum the intercept must be less than ½ the on-column equivalent of the reporting limit.

11.3.2 Linear Calibration Using Average Calibration Factors

11.3.2.1 External standard calibration using average calibration factors involves the comparison of instrument response (e.g., peak area or peak height) from the target compounds in the sample to the responses of the target compounds in the calibration standards. The ratio of the detector response to the concentration of target analyte in the calibration standard is defined as the calibration factor (CF), as follows:

$$CF = \frac{R_x}{C_s} \qquad \textit{Equation 3}$$

Where: R_x = Response for analyte
C_s = Concentration in calibration standard, µg/mL

11.3.2.2 For each target analyte, calculate the average calibration factor ($\overline{CF}$) as follows:

$$\overline{CF} = \frac{\sum_{i=1}^{n} CF_i}{n} \qquad \textit{Equation 4}$$

Where: n = Number of calibration levels
CF_i = Calibration factor for the i^{th} level

11.3.2.3 The calibration relationship can be graphically represented as a line through the origin with a slope equal to the average calibration factor.

11.3.2.4 The relative standard deviation (RSD) is calculated as follows:

$$RSD = \frac{SD}{\overline{CF}} x100\% \qquad \textit{Equation 5}$$

Where SD is the standard deviation of the average CF, which is calculated as follows:

$$SD = \sqrt{\frac{\sum_{i=1}^{n} \left(CF_i - \overline{CF}\right)^2}{n-1}} \qquad \textit{Equation 6}$$

11.3.2.5 To calculate the concentration in an unknown sample extract,

the equation is solved for concentration, resulting in the following equation:

$$C_{ex} = \frac{R_x}{\overline{CF}} \qquad \textit{Equation 7}$$

Where:
- C_{ex} = Extract analyte concentration, µg/mL
- R_x = Response for analyte
- $\overline{CF}$ = Average Calibration Factor

11.3.3 Average Calibration Factor Evaluation

Examine the residuals, i.e., the difference between the actual calibration points and the plotted line. Particular attention should be paid to the residuals for the highest points, and if the residual values are relatively large, a linear regression should be considered.

Acceptance Criteria: The RSD of the average response factor must be <20%. (15% for Method 8330B for DOD/DOE QSM 5.0 and 5.1). Also examine the residuals, especially for the high points versus the fitted function. If the residual values are large, a linear regression should be considered.

Corrective Action: If the RSD is > 20% (or >15% for Method 8330B for DOD/DOE QSM 5.0 and 5.1), average response factor cannot be used and least-squares linear regression should be attempted.

11.3.4 Linear Calibration Using Least-Squares Regression

Calibration using least-squares linear regression produces a straight line that does not pass through the origin. The calibration relationship is constructed by performing a linear regression of the instrument response (peak area or peak height) versus the concentration of the standards. The instrument response is treated as the dependent variable (y) and the concentration as the independent variable (x).

The weighting used is the reciprocal of the concentration or the reciprocal of the square of the concentration. The regression produces the slope and intercept terms for a linear equation in the following:

$$R_x = m_1(C_s) + b \qquad \textit{Equation 8}$$

Where:
- C_s = Concentration in calibration standard, µg/mL
- R_x = Response for analyte
- b = y - Intercept
- m_1 = Slope

To calculate the concentration in an unknown sample extract, the regression equation is solved for concentration, resulting in the following equations, where C_{ex} is the concentration of the target analyte in the unknown sample:

$$C_{ex} = \frac{[R_x - b]}{m_1}$$ *Equation 9*

Where: C_{ex} = Extract analyte concentration, µg/mL
R_x = Response for analyte
b = y - Intercept
m_1 = Slope

11.3.5 Evaluation of the Linear Least-Squares Regression Calibration Function:

With an unweighted linear regression, points at the lower end of the calibration curve have less weight in determining the curve than points at the high concentration end of the curve. For this reason, inverse weighting of the linear function is recommended to optimize the accuracy at low concentrations.

Note that the August 7, 1998 EPA memorandum "Clarification Regarding Use of SW-846 Methods", Attachment 2, Page 9, includes the statement "The Agency further recommends the use of this for weighted regression over the use of an unweighted regression."

Acceptance Criteria:

11.3.5.1 Examine the residuals, but with particular attention to the residuals at the bottom of the curve. If the intercept or the residuals are large, a second-order regression should be considered.

11.3.5.2 The linear regression must have a correlation coefficient (r) ≥ 0.995 (r^2 ≥ 0.990).

11.3.6 Non-linear Calibration Using a Second-Order Equation

When the instrument response does not follow a linear model over a sufficiently wide working range, or when the previously described calibration approaches fail acceptance criteria, a non-linear, second-order calibration model may be employed. The second-order calibration uses the following equation:

$$R_x = m_2(C_s)^2 + m_1(C_s) + b$$ *Equation 10*

Where: C_s = Analyte concentration in calibration standard, µg/mL

R_x	=	Response for analyte
m_2	=	Curvature
m_1	=	Slope
b	=	y - Intercept

To calculate the concentration in an unknown sample extract, the roots of the quadratic equation are solved for:

$$C_{ex} = \frac{-m_1 \pm \sqrt{(m_1)^2 - 4(m_2)(b - R_x)}}{2m_2}$$ *Equation 11*

Where:

C_{ex}	=	Extract analyte concentration, µg/mL
R_x	=	Response for analyte
m_2	=	Curvature
m_1	=	Slope
b	=	y – Intercept

11.3.7 Evaluation of Second-Order Regression Calibration:

A minimum of six points must be used for a second-order regression fit.

Acceptance Criteria:

11.3.7.1 Second-order regressions should be the last option, and note that some programs (e.g., South Carolina) do not allow the use of second-order regressions.

11.3.7.2 The coefficient of determination (COD, r^2) must be ≥ 0.99.

11.3.7.3 The response increases significantly with increasing standard concentration (i.e., the instrument response does not plateau at high concentrations).

11.3.7.4 The distribution of concentrations is adequate to characterize the curvature.

11.4 Calibration Verification

11.4.1 Initial Calibration Verification (ICV)

A second-source verification standard must be analyzed with each initial calibration. The calculated concentration of the analytes in this standard may not be greater than 20% different from the calibration standard (15% for DoD/DOE QSM 5.0 and 5.1 for Method 8330A).

11.4.2 Continuing Calibration Verification (CCV)

The working calibration curve or RF must be verified by the analysis of a mid-point continuing calibration standard at the beginning of the analysis sequence, after every 10 samples, and at the end of the analysis sequence.

Acceptance Criteria:

Results are acceptable for any individual compound if the %D (percent difference between the standard and measured values of the CCV standard) is ≤ 20%. (≤ 15% for DoD/DOE QSM 5.0 and 5.1 for Method 8330A). TestAmerica discourages the use of grand mean for method 8000B. The use of grand mean is not acceptable for Method 8000C (required by Arizona) or 8000D (required by North Carolina, South Carolina and West Virginia).

NOTE: In order to comply with DoD/DOE QSM 5.0 or 5.1 requirements, the use of the grand mean is not acceptable (refer to policy DV-QA-024P). Results are acceptable for individual compounds if the %D is:

8330A		8330B	
QSM 4.2	%D ≤ 15%	QSM 4.2	%D ≤ 20%
QSM 5.0	%D ≤ 15%	QSM 5.0	%D ≤ 20%
QSM 5.1	%D ≤ 15%	QSM 5.1	%D ≤ 20%

Corrective Action:

If the percent difference for any analyte falls outside of ± 20% (or program specific limit such as DoD or DOE), corrective action must be taken. This may include back flushing the column, changing the guard cartridge, changing the frit on the front end of the column, or other minor instrument adjustments, followed by reanalyzing the standard. If the response for any analyte still varies by more than 20% (or program specific limit such as DoD or DOE), a new calibration curve must be prepared and analyzed. The column may also need to be replaced based on the chromatography.

Reported sample results must be bracketed by successful CCVs. When a CCV fails, all samples run since the last successful calibration verification must be reanalyzed. If the CCV recovery is >20% D (or program specific limit such as DoD or DOE) and the associated samples are ND, the samples may be reported without reanalysis. Flag the data and document the decision in an NCM. For DoD or DOE, this must be accepted by the client and documented in the project records.

12.0 Calculations / Data Reduction

12.1 Detailed calibration equations can be found in the corporate SOP CA-Q-P-003, *Calibration Curves and the Selection of Calibration Points* and under the public folder, Arizona Calibration Training.

12.2 Qualitative Identification

12.2.1 Tentative identification occurs when a peak is found within the retention time window for an analyte, at a concentration above the method detection limit. The required quantitation level is defined as the LIMS test code reporting limit for standard reports, adjusted for initial weight and volume and any dilutions. A UV detector wavelength of 254 nm is used to quantify and report all analytes except PETN and Nitroglycerin that are quantified and reported using a UV detector wavelength of 215 nm.

12.2.2 Identification is confirmed if a peak is also detected within the retention time window on a dissimilar column (Section 12.3).

12.2.3 Sample Evaluation:

12.2.3.1 Analyst judgment weighs heavily in the evaluation of retention time shifts for client samples. The evaluation may be based on RT shifts of the surrogate standard. The chromatograms must be examined closely to ensure that false positive / negative results are not reported. In the absence of significant shifts of the surrogate, peaks within a ± 0.035 minutes (C18 column) or ± 0.07 minutes (phenyl hexyl column) window must be considered positive results.

12.2.3.2 If the sample required significant dilution due to high levels of target peaks or interfering compounds, the surrogate peak may not be obvious. In this case, an adjustment of RTs due to matrix for target compounds cannot be done reliably, and ± 0.035 minutes (C18 column) or ± 0.07 minutes (phenyl hexyl column) (or the established RT window described in Section 10.5.7) from the most recent CCV will be used for all compounds.

12.2.3.3 The expected retention time for target analytes is updated with the retention times of each CCV. If sample matrix is causing significant retention time shifts between CCVs, samples may require dilution and reanalysis to minimize the matrix effects.

12.2.3.4 Method Blank Detections:

When a detection is observed in the method blank on either column, the result must be confirmed on the other dissimilar column to be considered a true hit. See Section 9.3 and

Appendix 4 for additional information for evaluation of method blanks.

12.2.3.5 Interferences:

2,4,6-Trinitrotoluene elutes closely with 4-amino-2,6-dinitrotoluene on the primary (C18) column. Because of this close elution, high levels of 2,4,6-trinitrotoluene can overlap the retention time window for, and thus mask the presence of, low levels of 4-amino-2,6-dinitrotoluene. Therefore, 4-amino-2,6-dinitrotoluene may be reported as a detection from the confirmation (phenyl-hexyl) column, even though no peak could be detected on the primary column. In this event, an NCM is necessary.

Other target compound interferences may be observed in samples. Analyst judgment will be necessary to evaluate sample chromatograms and potentially report additional detections without confirmation due to interferences and/or analyze samples at dilutions. In this event, an NCM is required, documenting the decision.

12.3 Second-Column Confirmation

Detection of compounds on the primary column is confirmed using a second, dissimilar column. This column is calibrated using the same calibration levels as the primary column. The analysis on the second column must meet all of the instrument QC described in Section 9.0 and 11.0. Identification is confirmed if a peak is also present in the retention time window for that analyte on the confirmatory column.

The RPD between two results is calculated using the following equation:

$$\%RPD = \frac{\left|R_1 - R_2\right|}{\frac{1}{2}\left(R_1 + R_2\right)} \times 100\% \qquad \textit{Equation 12}$$

Where R_1 is the result for the first column and R_2 is the result for the second column.

Acceptance Criteria:

The RPD between confirmed results should agree within 40%; results will then be reported from the primary column.

Corrective Action:

If the RPD is >40% and there is visible positive interference, e.g., co-eluting peaks, elevated baseline, etc., for one column and not the other, then report the results from the column without the interference with the appropriate data qualifier flag, footnote, and/or narrative comment in the final report.

If the RPD is >40% and there is visible positive interference for both columns, then report the lower of the two results with the appropriate flag, footnote, and/or narrative comment in the final report.

Special project reporting requirements may supersede these reporting schemes. Verify in the method comments or project Quality Assurance Summaries.

12.4 Manual Integrations

Raw instrument data is automatically transferred to Chrom at the completion of each run for further processing. Review the chromatograms to ensure correct assigning of peaks and correct integration of each peak. If manual data manipulations are necessary, they must be justified and documented. See DV-QA-011P for requirements for manual integration.

12.5 % Difference Calculation for ICV / CCV Evaluation

The percent difference for the analysis of a CCV standard is calculated as follows:

$$\%\ \text{Difference} = \left(\frac{\text{Expected Value} - \text{Measured Value}}{\text{Expected Value}} \right) \times 100\% \qquad \textit{Equation 13}$$

12.6 Concentration in Aqueous Samples

The concentration of analyte in the original aqueous sample is calculated as follows:

$$\text{Concentration}, \mu\text{g/L} = \frac{C_{ex} V_t}{V_o} xDF \qquad \textit{Equation 14}$$

Where:
- C_{ex} = Extract analyte concentration, µg/mL
- V_t = Volume of total extract in mL (normally 5 mL)
- V_o = Volume of water extracted in liters (normally 0.5 L)
- DF = Dilution factor, as appropriate

12.7 Concentration in Soil Samples

The concentration of analyte in the original non-aqueous sample is calculated as follows:

$$\text{Concentration, } \mu\text{g/kg} = \frac{C_{ex}V_t}{WD} \times DF \qquad \textit{Equation 15}$$

Where:
- C_{ex} = Concentration of analyte in the extract (µg/mL)
- V_t = Total volume of original extract in mL (normally 20 mL for 8330A or 40 mL for 8330B); this volume includes the extracted volume in ACN and the 1:1 dilution with $CaCl_2$
- W = Weight (mass) of sample extracted in kg (normally 0.002 kg for 8330A or 0.010 kg for 8330B)
- D = (100-% moisture in sample)/100 for dry weight basis or 1 for wet-weight basis
- DF = Dilution factor, as appropriate

12.8 Concentration of Ammonium Picrate

Ammonium picrate is requested by some clients as a target compound. This is the ammonium salt of picric acid. Chromatographically, there is no difference between these two compounds, therefore, upon request, the result for ammonium picrate (C_{AP}) is calculated based on the measured concentration of picric acid, as follows:

$$C_{AP} = Picric\ Acid\ Result \times \left(\frac{246.13\,(Molar\ Mass\ of\ \ Ammonium\ Picrate)}{229.11\,(Molar\ Mass\ of\ \ Picric\ Acid)} \right) \qquad \textit{Equation 16}$$

In TALS, this calculation is accomplished by opening the batch information in the analytical batch, and setting the calculation line to 1 (Yes).

12.9 LCS and CCV Percent Recovery

$$\text{Control Spike Recovery} = \frac{S_{SR}}{S_A} \times 100\% \qquad \textit{Equation 17}$$

Where
- S_{SR} = Calculated analyte concentration of spiked sample
- S_A = Concentration of standard added

12.10 MS / MSD Percent Recovery Calculation

$$\text{Matrix Spike Recovery} = \frac{S_{SR} - S_R}{S_A} \times 100\% \qquad \textit{Equation 18}$$

Where
- S_{SR} = Calculated analyte concentration of spiked sample
- S_R = Calculated analyte concentration of parent sample
- S_A = Concentration of standard added

12.11 Relative Percent Difference Calculation for the MS/MSD

$$RPD = \frac{\left|MS_R - MSD_R\right|}{1/2(MS_R + MSD_R)} \times 100 \qquad \textit{Equation 19}$$

Where RPD = Relative percent difference
MS_R = Matrix spike result of analyte
MSD_R = Matrix spike duplicate result of analyte

12.12 Reporting limits are shown in Table 1. If samples require dilutions or smaller volumes than normally used, the MDLs and RLs will be correct based on the actual volume used and/or the dilution factor. Reporting limits for soil samples are adjusted for the actual weight of sample extracted. As samples are dried prior to subsampling for analysis percent moisture is not determined.

12.13 All results are subject to two levels of technical review. See SOP DV-QA-0020 for a more detailed description for data review and an example of this checklist.

13.0 Method Performance

13.1 Method Detection Limit Study (MDL)

The method detection limit (MDL) is the lowest concentration that can be detected for a given analytical method and sample matrix with 99% confidence that the analyte is present. The MDL is determined according to the laboratory's MDL policy in DV-QA-005P. MDLs reflect a calculated (statistical) value determined under ideal laboratory conditions in a clean matrix, and may not be achievable in all environmental matrices. The laboratory maintains MDL studies for analyses performed; these are verified at least annually unless method or program requirements require a greater frequency.

13.2 Demonstration of Capabilities

All personnel are required to perform an initial demonstration of proficiency (IDOC) on the instrument they will be using for analysis prior to testing samples. On-going proficiency must be demonstrated annually. IDOCs and on-going proficiency demonstrations are conducted as follows.

13.2.1 Four aliquots of the QC check sample are analyzed using the same procedures used to analyze samples, including sample preparation. The concentration of the QC check sample should be equivalent to a mid- level calibration.

13.2.2 Calculate the average recovery and standard deviation of the recovery for each analyte of interest

13.2.3 If any analyte does not meet the acceptance criteria, the test must be repeated. Only those analytes that did not meet criteria in the first test need to be evaluated. TNI 2009 requires consecutive passing results. Repeated failure for any analyte indicates the need for the laboratory to evaluate the analytical procedure and take corrective action.

13.2.4 Until the IDOC is approved by the QA Manager (or designee); the trainer and trainee must be identified in the batch record.

13.2.5 Further details concerning demonstrations of proficiency are described in SOP DV-QA-0024.

13.3 Training Requirements

The Group Leader is responsible for ensuring that this procedure is performed by an associate who has been properly trained in its use and has the required experience. A new analyst must be working under documented supervision prior to approval of the IDOC. Documentation that a new analyst is performing under supervision must be entered into the batch record (View Batch Information) until that analyst's IDOC has been approved by the QA Manager (or designee). See requirements for demonstration of analyst proficiency in SOP DV-QA-0024.

14.0 Pollution Control

Standards and reagents are prepared in volumes consistent with laboratory use to minimize the volume of expired standards and reagents requiring disposal.

15.0 Waste Management

15.1 All waste will be disposed of in accordance with Federal, State, and local regulations. When reasonably feasible, technological changes have been implemented to minimize the potential for pollution of the environment. Employees will abide by this procedure, the policies in section 13, "Waste Management and Pollution Prevention", of the Corporate Environmental Health and Safety Manual, and DV-HS-001P, "Waste Management Program."

15.2 The following waste streams are produced when this method is carried out:

15.2.1 Expired Chemicals/Reagents/Standards – Contact Waste Coordinator

15.2.2 Flammable solvent waste – Waste Stream C

15.2.3 Flammable vial waste – Waste Stream A

NOTE: Radioactive and potentially radioactive waste must be segregated from non-radioactive waste as appropriate. Contact the Radioactive Waste Coordinator for proper management of radioactive or potentially radioactive waste generated by this procedure.

16.0 References / Cross-References

16.1 SW-846, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, Third Edition and all promulgated updates, EPA Office of Solid Waste, January 2005.

16.1.1 Method 3535A, Solid Phase Extraction (SPE), Revision 0, December 1996.

16.1.2 Method 8000B, Determinative Chromatographic Separations, Revision 2, December 1996.

16.1.3 Method 8000C, Determinative Chromatographic Separations, Revision 3, March 2003.

16.1.4 Method 8000D, Determinative Chromatographic Separations, Revision 4, July 2014.

16.1.5 Method 8330, Nitroaromatics and Nitramines by High Performance Liquid Chromatography, Revision 0, September 1994.

16.1.6 Method 8330A, Nitroaromatics and Nitramines by High Performance Liquid Chromatography, Revision 1, January 1998.

16.1.7 Method 8330B, Nitroaromatics, Nitramines, and Nitrate Esters by High Performance Liquid Chromatography, Revision 2, October 2006.

16.2 Department of Defense Quality Systems Manual for Environmental Laboratories, Final Version 4.2, 10/25/2010.

16.3 Department of Defense/Department of Energy Consolidated Quality Systems Manual for Environmental Laboratories Version 5.0, July 2013.

16.4 Department of Defense/Department of Energy Consolidated Quality Systems Manual for Environmental Laboratories Version 5.1, 2017.

17.0 Method Modifications:

17.1 Deviations from Method Source and Rationale

Method 8330 prescribes the shelf life for standards as follows:

Standards	Concentration	Shelf Life per 8330A	Shelf Life per 8330B
Stock standards	1,000,000 µg/L (1,000 ppm)	Six Months	One year
Intermediate standards	2.5 to 10,000 µg/L	Thirty days	One year
Working standards	1 to 500 µg/L	Daily	Daily

This SOP describes the use of 100,000 µg/L high-level standards, which are assigned a six month shelf life based on TestAmerica's experience with these materials. Further, a 25-1,000 µg/L standard mix is characterized as an intermediate-level, and assigned a 30 day shelf life.

17.2 Acidic water (pH < 3) is added to the concentrated extract, dilutions, and all calibration and check standards in place of reagent water. This is to preserve any Tetryl present in the extract.

17.3 Method 8330B suggests that the %RSD for triplicate analysis for soil should be ≤10%. The laboratory uses a criterion of ≤ 20% consistent with DoD/DOE QSM requirements.

18.0 Attachments

Appendix 1. Analyte List
Appendix 2. Suggested Calibration Levels (µg/mL)
Appendix 3. Spike Levels
Appendix 4. Assessment of Method Blank Results
Appendix 5. Suggested Instrument Conditions
Appendix 6. Example Chromatogram from Primary Column – Ultracarb ODS (20)
Appendix 7. Example Chromatogram from Confirmation Column – Luna Phenyl-Hexyl

19.0 Revision History

- Revision 19, dated 30 June 2017
  - Updated text and references to DOD QSM to DOD/DOE QSM 5.0 and/or 5.1 as appropriate throughout SOP.
  - Revised low point of curve in Sections 1.5.2 and 7.5.2 and Appendix 2.
  - Added statement to Section 4.3 that both DOD/DOE QSM 5.0 and 5.1 exclude vegetation.
  - Updated reference from 2003 NELAP Standard to 2009 TNI Standard in Section 9.4.
  - Removed language in Section 9.5 regarding dilution of MS/MSD and parent sample as how to handle over-range MS/MSD is discussed later in the paragraph.
  - Added DOD/DOE QSM 5.1 requirements for grinding blank to Section 9.9.
  - Added notes regarding states requiring 8000D (North Carolina, South Carolina, West Virginia).
  - Added reference to 8000D in Section 16.
  - Updated Section 17.1 to reflect current shelf life for standards
  - Added 3,5-Dinitroaniline (Method 8330B only) to Appendix 2.

- Revision 18, dated 6 July 2016
  - Added clarification to Section 1.3 to comply with document control policy
  - Revised Section 7.8.10 to require no changes to eluent composition between initial calibrations.
  - Removed reference to DoD QSM 3 in Section 8.2.1. The laboratory no longer performs work in compliance with this outdated version of the QSM.
  - Added reference to grinding blank in Method Blank description in Section 9.3
  - Clarified corrective action for failed method blank in Section 9.3
  - Added statement to Section 9.4 to analyze LCS on confirmation column if samples analyzed on confirmation column (positive control).
  - Revised corrective action for Section 9.5 to include requirements for dilution of MS/MSD when required
  - Added clarification to replicate analyses in Section 9.8

- Removed section 9.11, redundant with Section 9 subsections.
- Added clarification for confirmation analyses in Section 10.5.3
- Added clarification in Sections 10.5.6.2 and 10.5.7.3 for updating RT windows only at start of each 12-hour sequence.
- Removed statement in Section 10.5.7.4 that RT can be adjusted based on each calibration verification standard.
- Added “at least annually” to section 10.5.7.5 for consistency with requirements in the QA Manual.
- Removed statements regarding changes to eluent between initial calibrations.
- Revised corrective action in Section 10.5.8.
- Added method reference to DoD QSM criteria stated in Section 11.3.3
- Removed paragraph referencing the grand mean; TestAmerica discourages the use of the grand mean.
- Clarified Section 12.2.3.4 on Method Blank Detections referencing Section 9.3 and new Appendix 4.
- Clarified sentence in Section 12.2.3.5 regarding interferences.
- Removed references in Section 12.2.3.5 to using MS confirmation as the laboratory uses HPLC analysis on a second distinct column for confirmation rather than MS analysis.
- Added sentence to Section 12.3 corrective action regarding potential for project specific reporting requirements pending results for dual column RPDs.
- Added section 15.2.3 for proper disposal of vial waste.
- Added Section 17.3 to the Method Modifications section.
- Added new Appendix 4 to provide MB interpretation guidance and renumbered remaining appendices.

- Revision 17, dated 31 March 2016
  - Revised Section 4.6 to clarify procedural steps to minimize decomposition of tetryl.
  - Added 12.0 mL vial size to section 6.2.1.1
  - Added TALS reagent IDs in Section 7
  - Revised preparation frequency of working eluent for primary column in Section 7.8.10.1
  - Revised amount of glacial acetic acid to add for working eluent for confirmation column, section 7.8.10.2
  - Added new section 9.6 to describe surrogate, acceptance criteria and corrective actions; renumbered remainder of section 9
  - Revised new sections 9.7 and 9.8 to clarify when duplicate and triplicate are required
  - Updated reference to corporate SOP for calibration curves from CA-Q-S-005 to current version CA-Q-P-003.
  - Revised Section 11.4.2 to clarify use of grand mean cannot be used in conjunction with Method 8000C such as required by Arizona and South Carolina
  - Revised equation 15 to include dry weight correction
  - Updated Section 13 to reflect current practice
  - Clarified DoD specific criteria throughout
  - Removed all references to AFCEE
  - Formatting and grammatical changes throughout.

- Revision 16, dated 31 March 2015
  - Annual Review
  - Updated section 1.4.4 to reflect current analyte exceptions

- Expanded and clarified section 7.1
- Made minor corrections to sections 7.3 and 7.4.
- Moved Instrument Maintenance and Troubleshooting section 13.5 to section 10.7
- Expanded the Instrument Maintenance and Troubleshooting section 10.7

- Revision 15, dated 31 March 2014
  - Updated Sections 2.2, 6.1.2, 7.8.10, Appendix 4 and Appendix 5 with a new primary column (Agilent Poroshell 120, EC-C18)
  - Added Section 13.5 as a DoD QSM 5.0 requirement
  - Added QSM reference information
  - Annual Review

- Revision 14, dated 30 April 2013
  - Updated Sections 9.1, 10.1 and 10.2
  - Annual Review

- Revision 13, dated 04 April 2012
  - Updated calibration section.
  - Updated standards section.
  - Replaced chromatograms in Appendices 5 and 6 with current chromatograms from Chrom.
  - Source method review.

- Revision 12.2, dated 15 February 2011
  - Added a comment to section 10.3 regarding saturation concentrations for the solid phase extraction cartridges.
  - Added section 11.1 referencing corporate SOP CA-Q-S005, “Calibration Curves”.

Earlier revision histories have been archived and are available upon request.

Appendix 1. Analyte List

Compound	Peak#		CAS #	Symbol	Standard Reporting Limits		
	Col A	Col B			Water (µg/L)	Soil, 2 g (mg/Kg)	Soil, 10 g (mg/Kg)
2,6-Diamino-4-nitrotoluene**	1	2	59229-75-3	2,6-DA-4-NT	1.0	2.0	1.0
Octahydro-1,3,5,7-tetranitro-1,3,5,7,-tetrazocine	2	5	2691-41-0	HMX	0.40	0.25	0.10
2,4-Diamino-6-nitrotoluene**	3	3	6629-29-4	2,4-DA-6-NT	1.0	2.0	1.0
Hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine	4	6	5755-27-1	MNX	2.0	0.25	0.10
Hexahydro-1,3,5-trinitro-1,3,5-triazine	5	7	121-82-4	RDX	0.20	0.26	0.20
Picric Acid (2,4,6-Trinitrophenol)	6	4	88-89-1	PA	2.0	0.25	0.10
1,3,5-Trinitrobenzene	7	18	99-35-4	1,3,5-TNB	1.0	0.25	0.10
1,2-Dinitrobenzene (surrogate)	8	9	528-29-0	1,2-DNB	NA	NA	NA
1,3-Dinitrobenzene	9	11	99-65-0	1,3-DNB	0.40	0.25	0.10
Methyl-2,4,6-trinitrophenyl nitramine	10	21	479-45-8	Tetryl	0.24	0.50	0.20
3,5-Dinitroaniline** (8330B only)	11	10	618-87-1	3,5-DNA	0.40	NA	0.10
Nitrobenzene	12	8	98-95-3	NB	0.40	0.25	0.30
Nitroglycerin	13	12	55-63-0	NG	3.0	5.1	2.0
2,4,6-Trinitrotoluene	14	22	118-96-7	2,4,6-TNT	0.40	0.25	0.10
4-Amino-2,6-dinitrotoluene	15	15	19406-51-0	4-Am-DNT	0.20	0.25	0.10
2-Amino-4,6-dinitrotoluene	16	17	35572-78-2	2-Am-DNT	0.20	0.25	0.10
2,6-Dinitrotoluene	17	19	606-20-2	2,6-DNT	0.20	0.25	0.10
2,4-Dinitrotoluene	18	20	121-14-2	2,4-DNT	0.40	0.25	0.10
2-Nitrotoluene (o-Nitrotoluene)	19	13	88-72-2	2-NT	0.40	0.25	0.20
4-Nitrotoluene (p-Nitrotoluene)	20	14	99-99-0	4-NT	1.0	0.40	0.20
PETN	21	23	78-11-5	PETN	2.0	4.0	2.0
3-Nitrotoluene (m-Nitrotoluene)	22	16	99-08-1	3-NT	0.40	0.50	0.20

**Non-standard spike analytes, only spiked when specifically requested.
A: - UltraCarb5uDODS
B: - Lina-Phenyl Hexyl

Appendix 2. Suggested Calibration Levels (µg/mL)

Compound	Level 1	Level 2	Level 3	Level 4*	Level 5	Level 6	Level 7	Level 8
HMX	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
RDX	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
1,3,5-Trinitrobenzene	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
1,3-Dinitrobenzene	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
Tetryl	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
Nitrobenzene	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
2,4,6-Trinitrobenzene	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
4-Amino-2,6-dinitrotoluene	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
2-Amino-4,6-dinitrotoluene	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
2,4-Dinitrotoluene	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
2,6-Dinitrotoluene	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
2-Nitrotoluene	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
3,5-Dinitroaniline (8330B only)	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
3-Nitrotoluene	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
4-Nitrotoluene	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
Nitroglycerin	0.2	0.5	1.0	2.5	4	7	10.	25
PETN	0.2	0.5	1.0	2.5	4	7	10.	25
2,4-Diamino-6-ditrotoluene	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
2,6-Diamino-4-nitrotoluene	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
Picric Acid	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5
1,2-Dinitrobenzene (surrogate)	0.02	0.05	0.10	0.25	0.4	0.7	1.0	2.5

* This level is used for the daily and continuing calibration standards.

Appendix 3. Spike Levels

LCS / MS / MSD Spike Levels					
Method and Matrix	Working Solution		Spike Amount	Final Concentrations	
	Standard Analytes	Nitroglycerin & PETN		Standard Analytes	Nitroglycerin & PETN
Water, Methods 8330A and 8330B	10 µg/mL	100 µg/mL	0.1 mL	2 µg/L	20 µg/L
Soil Method 8330A (2 g prep)	10 µg/mL	100 µg/mL	0.5 mL	2.5 mg/Kg	25 mg/Kg
Soil Method 8330B (10 g prep)	10 µg/mL	100 µg/mL	1.0 mL	1.0 mg/Kg	10 mg/Kg

Surrogate Spike Levels			
Method and Matrix	Working Solution 1,2-DNB	Spike Amount	Final Concentration
Water, Methods 8330A and 8330B	10 µg/mL	0.1 mL	2 µg/L
Soil Method 8330A (2 g prep)	10 µg/mL	0.5 mL	2.5 mg/Kg
Soil Method 8330B (10 g prep)	10 µg/mL	1.0 mL	1.0 mg/Kg

Appendix 4. Assessment of Method Blank Results

Primary Column	Confirmation Column	Corrective Action
NO	NO	Proceed with analysis; MB is ND
YES	NO	Proceed with analysis; MB is ND
NO	YES	Proceed with analysis; MB is ND
J FLAG	NO	Proceed with analysis; MB is ND
NO	J FLAG	Proceed with analysis; MB is ND
J FLAG	J FLAG	Detection confirms; however, no reworks needed if primary result < ½ RL If primary result > ½ RL, re-extract if possible unless the sample-samples are ND or >10x the MB for the analyte detected in the MB. Report with NCM
YES	YES	Samples must be ND or >10x the MB; otherwise re-extract. Report with NCM
J FLAG	YES	If primary result > ½ RL, re-extract if possible unless the sample analytes are ND or >10x the MB for the analyte detected in the MB. The relative percent difference between the primary and confirmation result determines which result to report. See Section 12.3. Flags applied by formatter. Report with NCM.
YES	J FLAG	

YES = Analyte was detected at a concentration above the RL
NO = Analyte was not detected above the MDL
J FLAG = Analyte was detected at a concentration less than the RL but at or above the MDL

NOTE: The formatter assigned in TALS will apply B flags according to program rules. Do NOT remove B flags. If a client requests such removal, QA staff and PM must be involved in the decision making process and all such removals must be documented. (See Policy P15-001.)

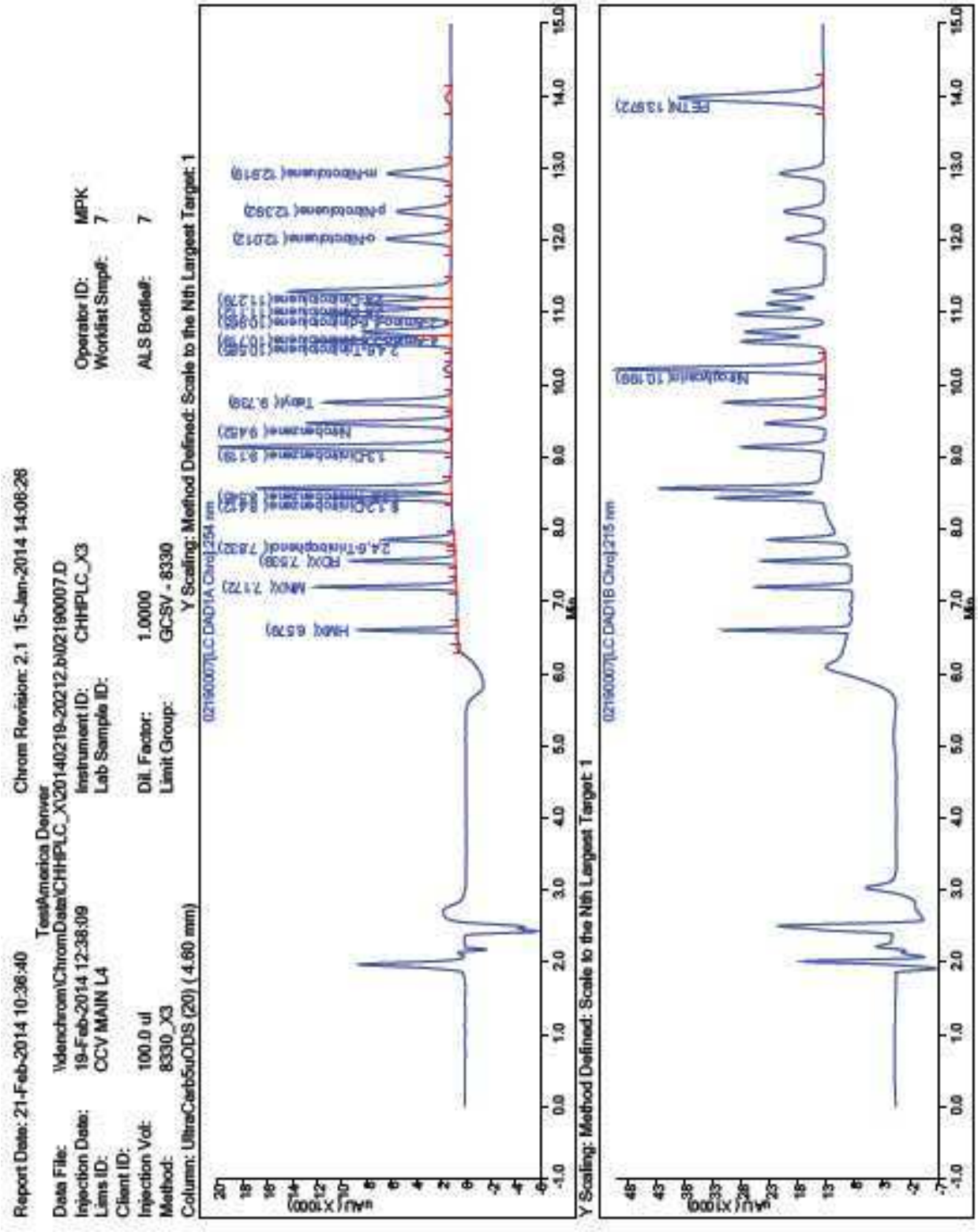
Appendix 5. Suggested Instrument Conditions

Instrument Conditions			
Column Types	Primary Column: Agilent Poroshell 120, EC-C18, 4.6 mm x 150 mm (2.7 µm)		
	Confirmation Column: Phenomenex Luna Phenyl-Hexyl, 4.6mm x 150mm (3.0 µm)		
Detector - 1st Channel	UV 254 nm, 40 R 550 nm		
Detector - 2nd Channel	UV 215 nm, 40 R 450 nm		
General Parameters		Primary Column	Confirmation Column
	Injection Volume:	50 µL	100 µL
	Column Temperature:	26.9℃	24.3℃

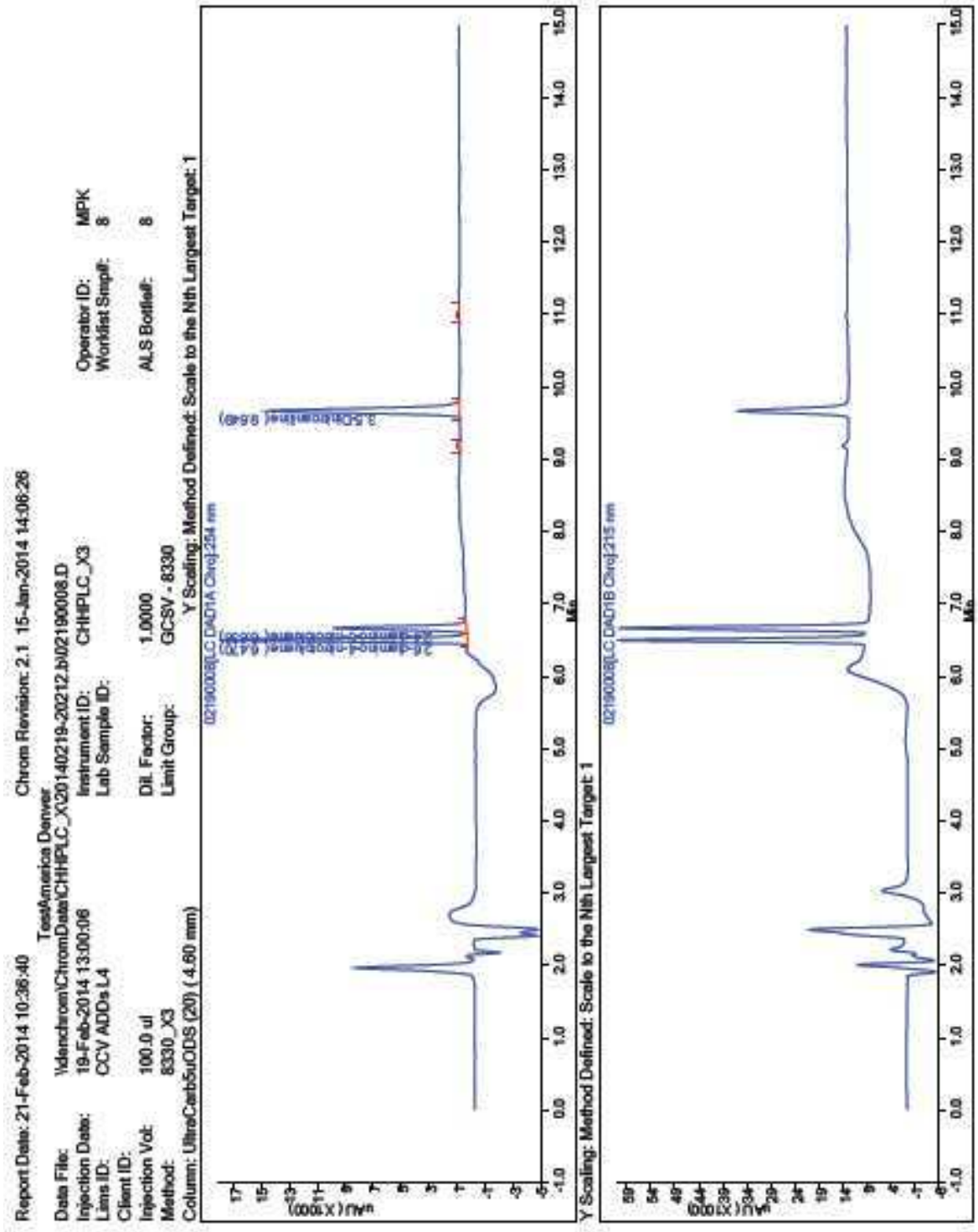
Suggested Column Parameters						
	Stop Time (min.)	**Post Time (min.)**	**Flow Rate (mL/min.)**	**Time (minutes)**	**% H_2O**	**% Methanol**
Gradient: Primary (C18) Column	15.5	5.0	0.75	0.0, 2.0, 2.98, 13.0, 13.5, 15.0, 15.5	90, 90, 41, 40,10, 10, 90	10, 10, 59, 60, 90, 90, 10
Gradient: Confirmation (Phenyl-Hexyl) Column	26.0	4.0	0.8	0.0, 26.0, 30.0	50, 25, 50	50, 75, 50

Appendix 6. Example Chromatograms from Primary (C18) Column

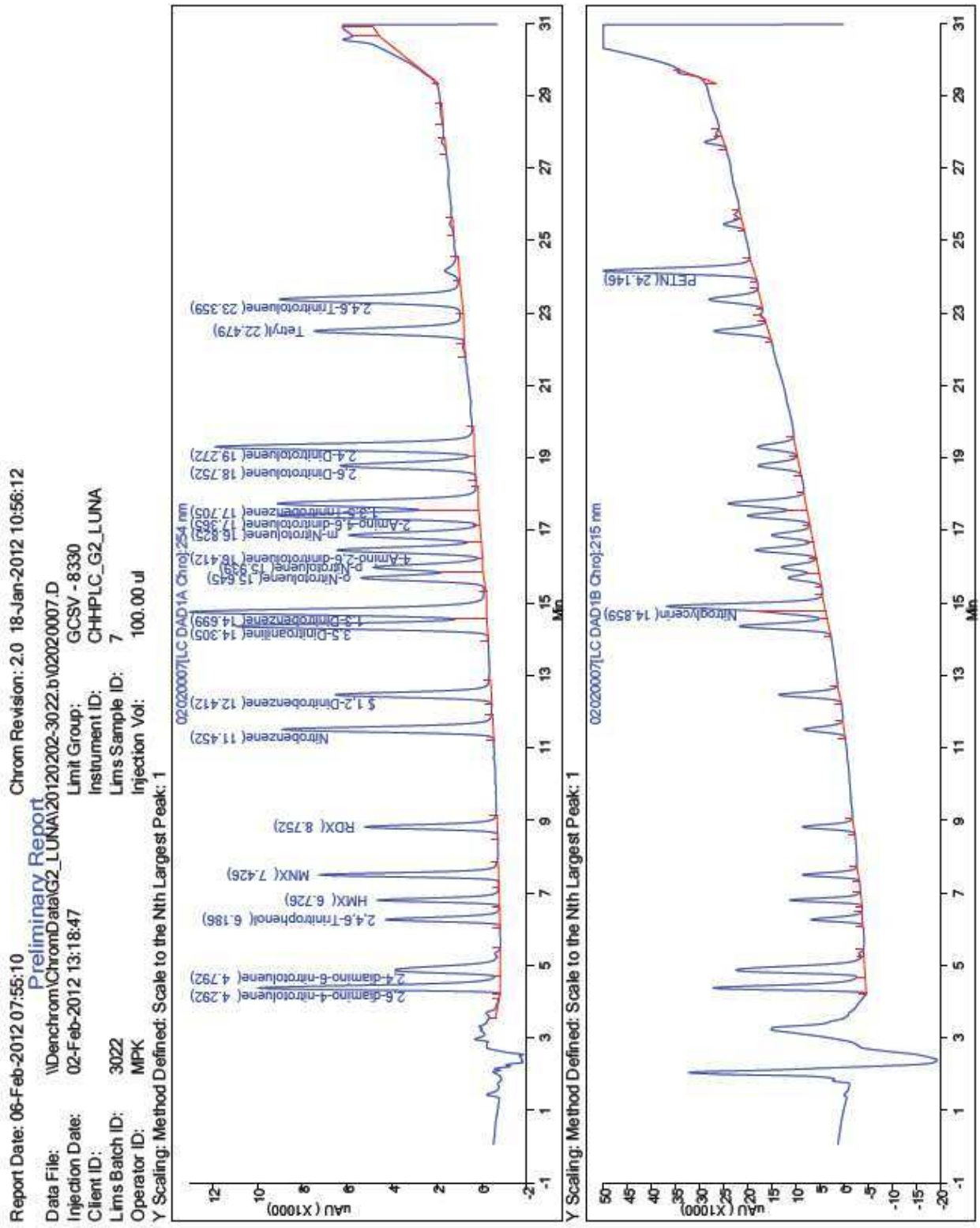
8330IntermStk:



Additional Compounds (8330_ADDs):



Appendix 7. Example Chromatogram from Confirmation (Phenyl-Hexyl) Column



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